Interactions of metabolic challenges with fertility in dairy cows

Geert Opsomer

Department of Reproduction, Obstetrics and Herd Health
Faculty of Veterinary Medicine
Ghent University
Belgium

Aims of the presentation

• To set the scene and indicate main metabolic challenges modern high yielding dairy cows are confronted with.

• Innovative research topics currently going on to unravel the pathophysiology of major metabolic stress in modern dairy cows especially focussing on the interaction with fertility problems.
High producing dairy cow

80Kg/day

Reproduction

Figure 2. Relationship between feed efficiency (milk/lb DM intake) and days in milk for 106 pairs of Holstein cows.
Major challenge for high milk production

- Availability of glucose:
  - 1 kg of milk: 72 g of glucose
  - 60 kg of milk: mammary glucose requirement: 4 kg/d
- Glucose mainly derived from gluconeogenesis
  - up 75% of glucose is made from propionate in the liver
Intake drop at calving means increased fat mobilization (Blood NEFA)

Dry Matter Intake Kg/day

Weeks relative to calving

Grummer, 1993

Glucose uptake by the mammary gland

- Major challenge for the cow since at that time dry matter intake is not maximal yet
- Maximal production only possible via absolute prioritization of glucose uptake by the mammary gland:
  - restriction of glucose consumption in peripheral tissues
  - maximization of (hepatic) glucose production
  - mobilization of energy reserves to provide alternative (=non-glucose) fuels for peripheral tissues
Adaptation towards high milk yield: Glucose-sparing state

- Similar for most pregnant/lactating mammals
- Lower insulin concentration
- Peripheral **insulin resistance**
- Result:
  - Lower expression GLUT-4 in muscle, fat
  - More glucose available for insulin-independent organs
    - Mammary gland
    - Gravid uterus
In vitro effect of elevated NEFA levels on proliferation of granulosa cells

- Fatty acids like palmitic and stearic acid have a significant negative effect on granulosa cell growth and proliferation

(Vanholder, 2005)

In vitro effect of elevated NEFA levels on oocyte and embryo quality

- Parameters: oocyte maturation, fertilisation rate, cleavage rate and blastocyst formation
- Palmitic and stearic acid: negative effect on all parameters, oleic acid: no effect

(Leroy, 2005)

Fat specificity of the dye Nile Red

(Leroy, 2005)
BHB and glucose

- Normal: 3.3 mM glucose and 0.3 mM BHB
- Subclinical ketonemia: 2.8 mM glucose and 1.8 mM BHB
- Clinical ketonemia: 1.4 mM glucose and 4.0 mM BHB

In vitro maturation model

Conclusions

Simultaneous exposure to low glucose and high BHB concentrations during oocyte maturation had a negative effect on cleavage and blastocyst formation. Especially low glucose rather than high BHB was responsible for the observed effect.

Energy Balance and Reproduction

Energy Balance

- Overconditioned cows
  - have a lower dry matter intake
  - larger fat depots to be broken down

Hence are at a higher risk to suffer from:
- metabolic diseases: ketosis, fatty liver, hypocalcemia
- inflammatory/infectious diseases
- fertility problems

Fat cow syndrome (Morrow, 1975)
Fat cow syndrome | Metabolic syndrome
---|---

The health consequences of obesity in men

- Cancer
- Gall bladder disease
- Kidney failure
- Stroke
- Heart failure

What is this Syndrome?
- Etiology – not fully elucidated
- Environment – Polygenic causes
- Insulin resistance – Hyperinsulinemia
- Hyperglycemia – IFG, IGT, T2DM
- Abd. Obesity (● WC, ● WC / ht ratio), ● BMI
- Hypertension, Endothelial Dysfunction (ED)
- Dyslipidemia (● TG, ● sLDL, ● HDL)
- Pro-inflammatory state (● CRP, TNF-α, IL-6)
- Pro-coagulant state (● PAI-1, ● Fibrinogen)
- Premature atherosclerosis
Evolution of Man

Hormones secreted by the Adipocytes

Leptin↓
- Energy expenditure
- Insulin sensitivity

TNF-α↓
- Energy expenditure
- Insulin sensitivity

IL-6↓
- Energy expenditure

Resistin
- Contradicting reports, possibly improvement of insulin sensitivity

Adiponectin↓
- Plasma glucose →
  - Mechanism?
  - Gluconeogenesis
  - FFA oxidation
  - Triglyceride synthesis via DAG

Many others

4/10/2015
Cytobrush:

Taking samples during insemination
Cytotape versus Cytobrush
- Similar PMNs %
- Same cellularity
- Cytobrush is more bloody
- Cytotape has better quality
(Bogado Pascottini et al., 2015)

Preliminary results
- Total of samples 1,496 inseminations
- SCE prevalence 14.5% (3% PMNs)
- Total conception rate 43.58%
SCE (+) 21.18%  P = < 0.05
SCE (-) 46.36%

Measuring insulin sensitivity
- Gold standard: hyperinsulinemic euglucemic clamp
  – the amount of glucose needed to infuse/time to keep glucose concentration constant following an insulin infusion
- Several surrogate indices: HOMA, Quicki, RQuicki, RQuicki BHB (D. Haarstich)
Insulin resistance

- Decreased biological response of insulin sensitive tissues

Insulin concentration

Dose-response curve

- Normal insulin response
- Decreased insulin sensitivity
- Decreased insulin responsiveness

Study design

- 9 healthy dairy cows selected based on BCS
  - 3-3.5 (n = 4)
  - 4-5 (n = 5)
- Dry, pregnant cows
- Fed according to NRC requirements
  - Mais silage
  - Hay
- HEC test 19 days before expected parturition date (min 16 and max 21 days)

HEC test

- Hyperinsulinemic
  - Constant insulin infusion to reach a steady state after 60 to 90 minutes (hyperinsulinemic state)
  - 4 consecutive insulin infusions: 0.1; 0.5; 2 and 5 mU/kg/min (140 minutes each)
- Euglycemic
  - Monitor blood glucose
  - Speed of glucose infusion adapted to keep blood glucose constant and normal
- Clamp
  - Steady state where insulin is in a steady state and no or minor changes are necessary to keep blood glucose constant
  - At least 30 minutes
Cows were relaxed
GIR = glucose infusion rate

SSGIR = steady state glucose infusion rate

SSIC = steady state insulin concentration

ISI = insulin sensitivity index

P < 0.05, P < 0.01, 0.05 < P < 0.1

BCS < 4 (n=4) vs BCS > 4 (n=5)

Not significant
Conclusion

- The glucose metabolism of precalving dairy cows with a BCS>4 is more insulin resistant compared to cows with a BCS<4
  - Decreased insulin sensitivity and decreased insulin responsiveness
  - Possible explanations
    - NEFA
    - Adipokines (adiponectin, leptin, resistin, TNFα, IL6)

Hyperlipidemia causes insulin resistance in cows

IVGTT 8 hours after infusion of tallow emulsion

![Graph](Pires et al., 2007)

Hyperlipidemia causes insulin resistance in cows

IVITT 8 hours after infusion of tallow emulsion

![Graph](Pires et al., 2007)
Not all fat depots are the same

- Fat depots:
  - Visceral depots are considered more dangerous than subcutaneous depots
  - Direct contact to the liver, higher possibility to produce adipokines, more sensitive for lipolysis, higher concentration of saturated fatty acids

Intra-abdominal fat is highly predictive of insulin sensitivity

Intra-abdominal fat area (cm$^2$)

About apples and pears

“Apple” vs. “Pear”
Omentum score 1: omentum is a rather thin peritoneal 'membrane' with clearly visible blood vessels

Omentum score 5: The omentum is that fat that there is no longer a 'net structure' is visible
Correlation body condition score – omental score

\[ r = 0.202 \]
\[ P = 0.085 \]

(Van Eetvelde, 2009)

Figure 1. Omental adipose tissue mass in cows with different body condition scores. M = moderate energy diet, L = low energy diet.

(Nikkah et al., 2010)

Not all fatty acids are the same

- Nefas:
  - saturated fatty acids are proven to be more toxic than unsaturated fatty acids
Multiple Factors May Drive Progressive Decline of β-Cell Function

- Hyperglycemia (glucose toxicity)
- Obesity
- Insulin resistance
- Protein glycation
- "Lipotoxicity" (elevated FFA, TG)

Adapted from Unger RH, Orci L. Biochim Biophys Acta. 2002;1585:202-212.

The hyperbolic relationship

- Insulin secretion vs. Insulin sensitivity
- Lean non-diabetic subjects
- Obese non-diabetic subjects
- (Obese) Type 2 diabetic patients

IVGTT in periparturient dairy cows

- NEFA: negatively correlated with AUC<sub>ins</sub> and Peak<sub>ins</sub>

Bossaert, 2010.
The Dutch Famine

Nov. 1944 – Apr. 1945

Effect of prenatal exposure to the Dutch famine on size and obesity at age 50 in men

<table>
<thead>
<tr>
<th>Born before famine</th>
<th>Exposed to famine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>in early pregnancy</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79.0</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>91.8</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>26.7</td>
</tr>
<tr>
<td>(kg/m²)</td>
<td></td>
</tr>
<tr>
<td>BMI &gt; 25 kg/m²</td>
<td>65 %</td>
</tr>
</tbody>
</table>

Reville et al., Am J Clin Nutr, 1998

HUMAN
Malnutrition during pregnancy
Nutrient restriction embryo/fetus
Birth weight
Long term health
Reproduction

DAIRY COW
High milk yield/ NEB during gestation
Nutrient restriction embryo/fetus
Birth weight
Long term health
Reproduction
### Effect of lactation on fetal wt (g)

<table>
<thead>
<tr>
<th></th>
<th>Lact</th>
<th>Nonlact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 28 fetus</td>
<td>0.11 ± 0.15</td>
<td>0.12 ± 0.11</td>
</tr>
<tr>
<td>Day 35 fetus</td>
<td>0.61 ± 0.11</td>
<td>0.80 ± 0.15</td>
</tr>
<tr>
<td>Day 42 fetus</td>
<td>1.64 ± 0.10</td>
<td>2.02 ± 0.11</td>
</tr>
</tbody>
</table>

(Lucy, 2010)

### Day 28 bovine fetuses

Cow 15  
Cow 34  
Cow 39  

7 mm

(Lucy, 2010)
IVGTT in neonatal calves

- IV insulin bolus (0.05 IU/kg)

- Glucose constantly at a higher level in HF calves

HF: Higher AUC<sub>ins</sub>
Lower ER<sub>gluc</sub> More insulin resistant

(Bossaert, 2010)
Take home message

- Modern cows and men seem to struggle with very similar diseases:
  - striking similarities between ‘metabolic syndrome’ in humans and ‘fat cow syndrome’ in high yielding dairy cows
- Exploring the knowledge in human medicine may help to improve our knowledge in veterinary medicine:
  - pathophysiology, treatment and prevention

Questions?